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METHOD FOR INCREASING HDL CHOLESTEROL LEVEL

by

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METHOD FOR INCREASING HDL CHOLESTEROL LEVEL

This invention relates generally to the field of immunotherapy and, more specifically, to methods of stimulating an immune response to cholesteryl ester transfer protein (CETP).

BACKGROUND OF THE INVENTION

Blood cholesterol levels have long been thought to correlate directly with risk of atherosclerotic cardiac disease, the leading cause of heart attacks. More recently, it has been appreciated that blood cholesterol is actually composed of two primary forms: the high density lipoproteins (HDL) and low density lipoproteins (LDL). Rather than being associated with the disease risk, high HDL levels are apparently inversely predictive. In fact, studies have now indicated that HDL has a direct action in protecting against atherosclerosis and may even promote atherosclerosis plaque regression.

Numerous factors are involved in regulating the level of cholesterol in the body. Cholesteryl ester transfer protein (CETP) is an enzyme responsible for transporting cholesterol esters (CE) from HDL to very low density lipoproteins (VLDL) and LDL. VLDL's are eventually converted into LDL. CETP accelerates specifically the exchange of lipid components between pro- and anti-atherogenic lipo protein tractions. In particular, there is a strong inverse correlation between the levels of CETP in the plasma and the levels of HDL cholesterol. CETP activity levels are elevated in individuals suffering from dietary or genetic hypercholesterolemia. Increased levels of CETP activity result in lowered levels of HDL. In contrast, individuals with deficiencies in CETP activity due to mutations in the CETP gene have markedly elevated HDL levels.

The immune systems of higher organisms developed as a means for protecting the individual against invasion by deleterious foreign materials such as viruses, bacteria and parasites. Cells of the immune system are able to distinguish between materials from the individuals own body (termed "self" materials) and foreign material, or antigens. When foreign material enters the body, the immune system mounts a response. Antibodies that specifically recognize and bind to the foreign material are produced (the antibody or humoral response.) In addition, T cells are mobilized to repel the foreign substance (the T cell or cellular response.) Materials which are recognized as self do not normally stimulate such responses except in certain pathological conditions, primarily auto-immune disease. Even where the presence of an endogenous protein is itself deleterious, the immune system cannot serve as a regulator if the material is recognized as self.

Because of HDL'S potentially beneficial effect in preventing atherosclerosis, there exists a need for methods which can be used to increase its level in the serum. Such methods should ideally be specific and reliable and involve as little invasion of the body as possible. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a method for increasing HDL cholesterol in a mammal by stimulating an immune response that inhibits the function of CETP. Such an immune response can be induced by immunizing with CETP or fragments of CETP (together termed "CETP Peptides") which contain an epitope capable of stimulating such a response. The peptides can be conjugated to a carrier, such as Keyhole Limpet Hemocyanin (KLH) or ovalbumin, in

order to increase immunogenicity. Adjuvants can also be administered.

In one embodiment, the fragments of CETP used to raise the antibody response are about ten to twenty amino acids in length and contain sequences homologous to the sequence in rabbit or human CETP.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a means to utilize the body's own immune system to lower CETP levels, thereby increasing the level of beneficial HDL cholesterol. The invention provides an effective method of raising HDL in the blood or more specifically, the serum. By utilizing the body's own immune system to increase HDL levels, the invention avoids the problems associated with the repeated administration of drugs, which have undesirable side effects.

According to the present invention, CETP peptide is administered to an appropriate individual in such a manner as to elicit an anti-CETP immune response. The CETP can be chosen to contain an epitope capable of stimulating an antibody or humoral response. Alternatively, the CETP can stimulate a cellular response, or other immune response. CETP peptides can be elected to contain B cell epitopes, sequences capable of stimulating the production of antibodies that specifically recognize and bind to the epitope. Alternatively, CETP peptides can be chosen which stimulate a T cell or more general immune response.

Individuals exhibiting, or at risk of exhibiting, low serum levels of HDL cholesterol are particularly appropriate for such treatment. Serum HDL levels can be determined using methods well-known in the

art (See Warnick, G.R. J.Lipid. Res., 19:65 (1978), for example, which is incorporated herein by reference). Serum HDL of less than about 30-35 mg/dl is considered low. Subjects exhibiting a serum HDL level below this level are particularly suitable for the treatment of the invention.

The protein or peptide to be administered can be all or part of the CETP protein, so long as the protein or peptide contains a B cell and/or T cell epitope. As used herein, "CETP peptide" is intended to include both the full length CETP amino acid sequence as well as fragments thereof. The peptides can have a sequence corresponding to or homologous to a mammalian CETP sequence. It will be appreciated that the peptide can differ from the native sequence to some extent so long as it is capable of inducing antibodies that inhibit the activity of CETP.

CETP is a 55 kD protein based on its amino acid sequence, but with post-translational modifications it has an apparent molecular weight of 66-74 kD. The human CETP mRNA sequence is available in Genbank (accession number M30185). The rabbit CETP mRNA sequence is available in Genbank (accession number M27486). The genbank sequences were translated using the MacVector software program (I.B.M., New Haven, Connecticut) to obtain the complete amino acids sequence of human and rabbit CETP.

Because CETP and its peptide derivatives may be recognized are "self" antigens, carriers can be used to increase their immunogenicity. Such carriers are well known in the art and include, for example, such compounds as Keyhole Limpet Hemocyanin (KLH), ovalbumin and Diphtheria toxoid (Wako BioProducts). The CETP peptides can be conjugated to such carriers by methods well-known

in the art. See Current Protocols in Molecular Biology, Ausebell, Brent, Kingston, Moore, Seidman, Smith & Strull eds. (1987), or manufacturers' instructions, which is incorporated herein by reference. The immunogenicity of the peptides can be also increased by administration of a adjuvant. Various adjuvants are well-known and available. See Antibodies: A Laboratory Manual, Harlow and Lane eds., (1988) which is incorporated herein by reference.

The extent of the anti-CETP response induced by the administration of the CETP peptides can be monitored using a variety of assays. For example, competitive format immunoassays can be employed using anti-CETP antibodies or anti-CETP antiserum. Alternatively, the activity level of the CETP in the subject individual can be monitored using, for example a ^{3}H-cholesterol oleate transfer assay. Lasuncion, M.A., et al. Biochem J., 270:441-449 (1990). Reduction in CETP activity is an indirect indication of the anti-CETP response.

The following examples are intended to illustrate but not limit the invention.

Example 1
Administration of CETP peptide immunogen

Peptides corresponding to portions of the human, rabbit and rabbit/human CETP were prepared according to standard peptide synthesis protocols. The following peptide sequences were prepared:

H-Cys-Asp-Ser-Gly-Arg-Val-Arg-Thr-Asp-Ala-Pro-Asp-OH
(SEQ ID No.: 1)

H-Cys-Asp-Ala-Gly-Ser-Val-Arg-Thr-Asn-Ala-Pro-Asp-OH
(SEQ ID No.: 2)

H-His-Leu-Leu-Val-Asp-Phe-Leu-Gln-Ser-Leu-Ser-OH.
(SEQ ID No.: 3)

The first peptide (SEQ ID 1) is taken from the Human CETP peptide sequence (residues 131-142 without signal peptide) from Smith and Barakat, Med. Sci. Res., 21:911-912 (1993), which is incorporated herein by reference. The second peptide (SEQ ID 2) is the corresponding rabbit sequence and differs by only 3 amino acids from the human.

The third peptide (SEQ ID 3) is common to both human and rabbit and is an epitope recognized by anti-CETP-monoclonal antibody which is neutralizing. Tall, A.R., J. Lipids Res., 34:1255-1257 (1993).

The peptides were conjugated to ovalbumin by the procedure of Current Protocols in Molecular Biology, supra. Of four New Zealand White rabbits, approximately four months of age, two were injected intramuscularly with 100 micrograms of the ovalbumin-conjugated human peptide (Seq. ID No.: 1) and CFA in PBS saline and two were injected with the equivalent human/rabbit peptide (Seq. ID No. 3). The animals were boosted twice at one month intervals with with the same peptides in IFA..

Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications can be made withouth departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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